Human Epidermal and Monocyte-Derived Langerhans Cells Express Functional $P2X_7$ Receptors

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Monocyte-derived dendritic cells (Mo-DC) express functional $P2X_7$ receptors; however, the expression of these receptors on tissue-derived dendritic cells including epidermal Langerhans cells (LC) is unknown. Using immunolabeling and flow cytometry, we demonstrated that $P2X_7$ was present on both human epidermal LC and monocyte-derived LC (Mo-LC), as well as on human keratinocytes. The ecto-ATPDase (CD39) was also present on LC, but not keratinocytes. The $P2X_7$ agonists, 2′- and 3′-0(4-benzoylbenzoyl) adenosine 5′-triphosphate (BzATP) or ATP, but neither adenosine 5′-diphosphate (ADP) nor uridine 5′-triphosphate (UTP), induced ethidium$^+$ uptake into these cells. Furthermore, ATP-induced ethidium$^+$ uptake into epidermal LC, Mo-LC and keratinocytes was inhibited by the specific $P2X_7$ antagonist, KN-62 (1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine). ATP-induced ethidium$^+$ uptake into Mo-LC and Mo-DC was 2- and 3-fold greater, respectively, than that for fresh monocytes. $P2X_7$ activation on LC induced downstream signaling events, as BzATP or ATP, but neither ADP nor UTP, induced shedding of the low-affinity receptor for IgE (CD23) from Mo-LC. This process was inhibited by KN-62. Finally, ATP-induced ethidium$^+$ uptake and CD23 shedding were impaired in Mo-LC obtained from subjects homozygous for the loss-of-function Glu-496 to Ala polymorphism in the $P2X_7$ receptor. These results demonstrate that human LC express functional $P2X_7$ receptors, and suggest a role for this receptor in the skin immune system.

Key words: adenosine triphosphate/adenosinetriphosphatase/dendritic cells/keratinocytes/purinergic P2/receptors
J Invest Dermatol 125:482–490, 2005

Purinergic receptors are classified into either P1 receptors, selective for adenosine, or P2 receptors, selective for adenosine 5′-triphosphate (ATP) and adenosine 5′-diphosphate (ADP), which are subdivided into P2X and P2Y receptors (Ralevic and Burnstock, 1998). Extracellular nucleotides have attracted considerable interest as important signaling molecules in relation to both dendritic cells (DC) (la Sala *et al*, 2003) and the skin immune system (Granstein, 2002; Holzer and Granstein, 2004). DC are central in the initiation of adaptive immune responses and there is considerable interest in understanding the factors that govern the various stages of DC migration, maturation, and activation (Cavanagh and Von Andrian, 2002). Epidermal Langerhans cells (LC) are a subtype of DC located within the epidermis where they play an important role in the skin immune system (Romani *et al*, 2003); however, the expression and function of purinergic receptors on epidermal LC remain unknown.

P2X receptors are ligand-gated cation channels with identical subunits assembled in a trimeric structure each with two transmembrane domains containing intracellular amino and carboxyl termini (North, 2002). Activation of $P2X_7$ by 2′- and 3′-0(4-benzoylbenzoyl) ATP (BzATP) or ATP opens a cation-selective channel allowing an influx of Ca^{2+} and ethidium$^+$, and an efflux of K^+ (Surprenant *et al*, 1996; Gargett *et al*, 1997). $P2X_7$ receptor function varies considerably between subjects (Lammas *et al*, 1997; Gu *et al*, 2000), explained in part by loss-of-function polymorphisms (Gu *et al*, 2001, 2004; Wiley *et al*, 2003; Skarratt *et al*, 2005). $P2X_7$ is predominantly expressed on cells from the hematopoietic lineages for erythrocytes, T and B lymphocytes, NK cells, monocytes, and macrophages (Hickman *et al*, 1994; Gu *et al*, 2000; Sluyter *et al*, 2004b). In addition, $P2X_7$ has been identified on monocyte-derived dendritic cells (Mo-DC) where it plays a role in antigen presentation (Mutini *et al*, 1999), apoptosis (Coutinho-Silva *et al*, 1999), cytokine release (Ferrari *et al*, 2000), and the shedding of CD23 (Sluyter and Wiley, 2002). ATP can also permeabilize human epidermal LC via a molecule that may be $P2X_7$ (Girolomoni *et al*, 1993); however, this study preceded the cloning of the *P2X7* gene, and the development of anti-$P2X_7$ antibodies and $P2X_7$ antagonists, and thus a specific role for $P2X_7$ was not established. We have used flow cytometric and

Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; 7AAD, 7-aminoactinomycin D; BzATP, 2′- and 3′-0(4-benzoylbenzoyl) ATP; DC, dendritic cells; DMSO, dimethyl sulfoxide; EC, epidermal cells; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte macrophage-colony stimulating factor; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; IL, interleukin; LC, Langerhans cells; mAb, monoclonal antibody; MFI, mean fluorescence intensity; Mo-DC, monocyte-derived dendritic cells; Mo-LC, monocyte-derived Langerhans cells; PBS, phosphate-buffered saline; PE, phycoerythrin; TGFβ1, transforming growth factor-β1; UTP, uridine 5′-triphosphate

pharmacological approaches to investigate whether human LC express functional P2X$_7$ receptors.

Results

Human epidermal Langerhans cells and keratinocytes express P2X$_7$ To determine whether epidermal LC express P2X$_7$, epidermal cell (EC) suspensions were prepared from freshly excised human skin, labeled with mAb, and examined by flow cytometry. CD1a$^+$ EC (epidermal LC) were found to express P2X$_7$ on the cell surface with a mean fluorescence intensity (MFI) of 21.9 $\pm$ 17.1 (n = 5), as well as cell surface CD39 (MFI of 538.1 $\pm$ 248.1, n = 5) (Fig 1). It was noted that P2X$_7$ was also present on CD1a$^-$ EC, which predominantly consist of keratinocytes, and that expressed P2X$_7$ with an MFI of 49.3 $\pm$ 29.5 (n = 5), but were negative for CD39 (Fig 1).

P2X$_7$ agonists induce ethidium$^+$ uptake into epidermal Langerhans cells and keratinocytes The effect of various extracellular nucleotides on ethidium$^+$ uptake into CD1a$^+$ EC was then studied. Uptake of ethidium$^+$ in the absence of nucleotide was minimal (Fig 2). The P2X$_7$ agonists, 200 μM BzATP and 1 mM ATP, induced the uptake of ethidium$^+$ with an MFI of 483.5 $\pm$ 259.0 and 357.1 $\pm$ 214.1, respectively (n = 6; Fig 2). In contrast, 1 mM ADP and 1 mM uridine 5′-triphosphate (UTP) failed to induce ethidium$^+$ uptake (MFI of 1.6 $\pm$ 2.8 and 1.6 $\pm$ 2.6, respectively, n = 3; Fig 2) with values similar to the uptake of ethidium$^+$ in the absence of nucleotide.

Similar results were also observed with CD1a$^-$ EC (keratinocytes). BzATP and ATP induced the uptake of ethidium$^+$ with an MFI of 1154.6 $\pm$ 621.8 and 1014.8 $\pm$ 454.9, respectively (n = 6; Fig 2), whereas ADP and UTP failed to induce ethidium$^+$ uptake with an MFI of 1.8 $\pm$ 1.6 and 1.3 $\pm$ 2.2, respectively (n = 3; Fig 2). Of note, both BzATP- and ATP-induced uptake of ethidium$^+$ into keratinocytes

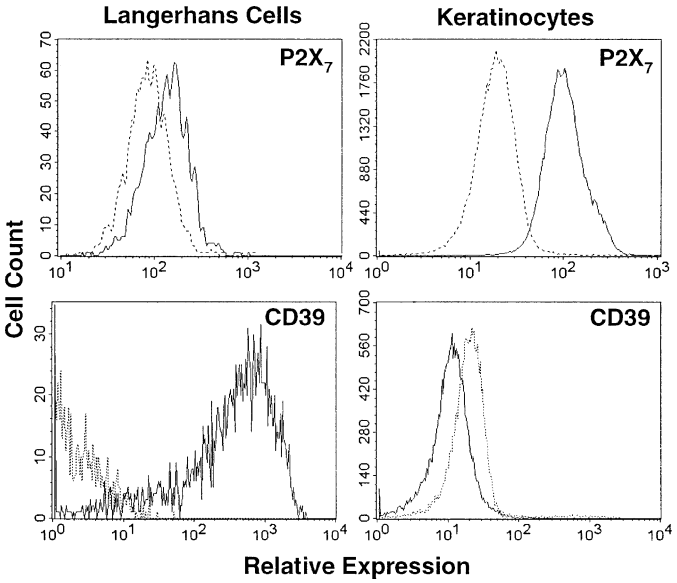

Figure 1
Expression of P2X$_7$ receptors and CD39 on epidermal Langerhans cells. Epidermal cells (EC) were labeled with fluorescein isothiocyanate (FITC)-P2X$_7$ (*solid line*) or FITC-isotype control (*dotted line*) monoclonal antibody (mAb), phycoerythrin (PE)-CD1a mAb and 7-aminoactinomycin (7AAD), or PE-CD39 (*solid line*) or PE-isotype control (*dotted line*) mAb, FITC-CD1a mAb and 7AAD, and analyzed by flow cytometry gating on CD1a$^+$7AAD$^-$ EC (Langerhans cells) and CD1a$^-$7AAD$^-$ EC (keratinocytes). Results are representative of five experiments.

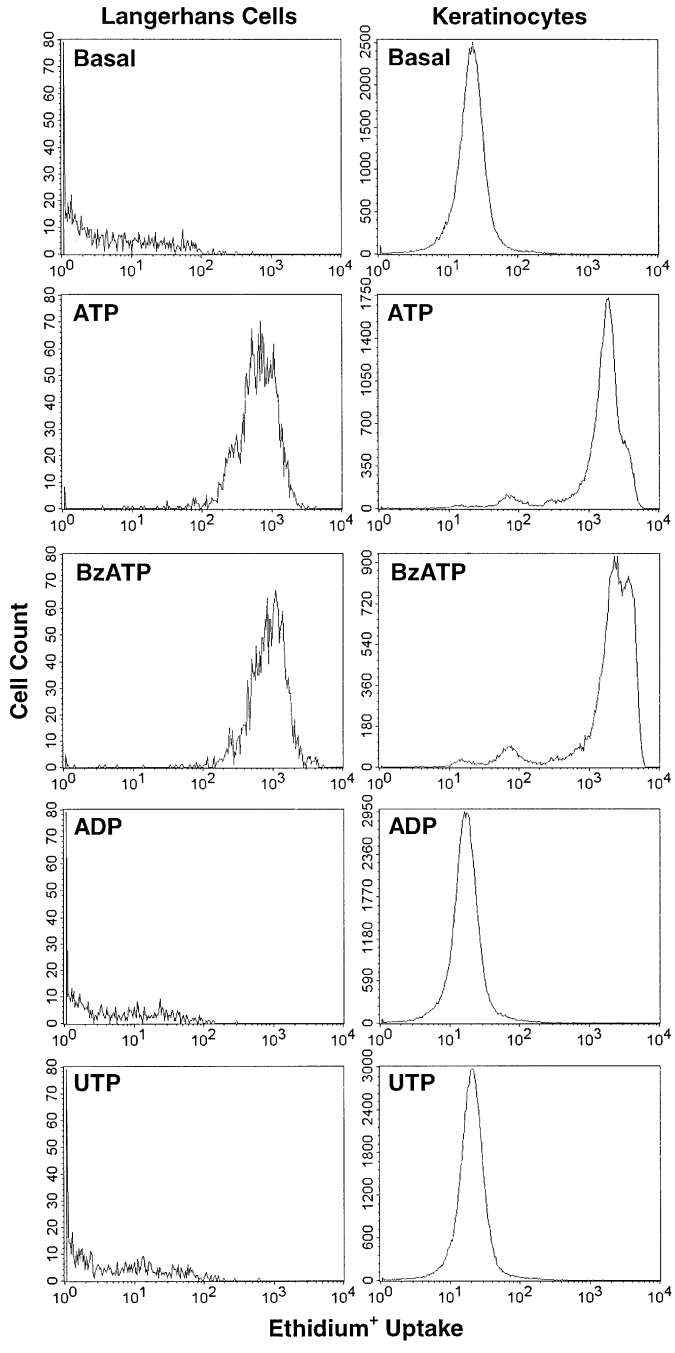

Figure 2
P2X$_7$ agonists induce ethidium$^+$ uptake into epidermal Langerhans cells (LC) and keratinocytes. Epidermal cells (EC) in KCl medium containing 25 μM ethidium$^+$ were incubated in the absence (basal) or presence of 1 mM ATP (adenosine 5′-triphosphate), 200 μM BzATP (2′- and 3′-0(4-benzoylbenzoyl) ATP), 1 mM ADP (adenosine 5′-diphosphate), or 1 mM UTP (uridine 5′-triphosphate) at 37°C for 5 min. Incubations were stopped by the addition of $MgCl_2$ medium followed by centrifugation. The EC were labeled with fluorescein isothiocyanate-CD1a mAb and analyzed by flow cytometry gating on CD1a$^+$ EC (LC) and CD1a$^-$ EC (keratinocytes). Results are representative of six (basal, ATP, and BzATP) or three (ADP and UTP) experiments.

Table I. $P2X_7$ antagonist inhibits ATP-induced ethidium$^+$ uptake into epidermal LC and keratinocytes

	ATP-induced ethidium$^+$ uptake (MFI)[a]			
	Epidermal LC		Keratinocytes	
DMSO	118.6 ± 45.9		543.5 ± 94.9	
1 μM KN-62	0 ± 0	$p<0.02$[b]	39.5 ± 13.8	$p<0.01$[b]
10 μM KN-62	0 ± 0	$p<0.02$[b]	6.0 ± 2.3	$p<0.01$[b]

[a]EC in KCl medium were pre-incubated with DMSO vehicle, 1 μM KN-62 or 10 μM KN-62 at 37°C for 15 min, before incubation with 25 μM ethidium$^+$ in the absence or presence of 100 μM ATP at 37°C for 5 min. Incubations were stopped by the addition of $MgCl_2$ medium followed by centrifugation. EC were labeled with FITC-CD1a mAb and analyzed by flow cytometry gating on CD1a$^+$ EC (epidermal LC) or CD1a$^-$ EC (keratinocytes). The level of ATP-induced ethidium$^+$ uptake was determined as the difference in MFI of cell-associated ethidium$^+$ of cells incubated in the presence and absence of ATP. Results are the mean ± SD of three experiments.

[b]To corresponding DMSO vehicle control.

ATP, adenosine 5′-triphosphate; EC, epidermal cells; LC, Langerhans cells; DMSO, dimethyl sulfoxide; KN-62, 1-[*N*,*O*-bis(5-isoquinolinesulphonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; MFI, mean fluorescence intensity

was significantly greater than that into epidermal LC ($p<0.05$ and $p<0.01$, respectively).

$P2X_7$ antagonist impairs ATP-induced ethidium$^+$ uptake into epidermal Langerhans cells and keratinocytes To examine a role for $P2X_7$ in the ATP-induced ethidium$^+$ uptake, EC were pre-incubated with the specific $P2X_7$ antagonist, KN-62 (1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine) (Gargett and Wiley, 1997), at either 1 or 10 μM or an equal volume of dimethyl sulfoxide (DMSO) vehicle, before incubation with ATP at a concentration of 100 μM, which is close to the EC_{50} (~ 85 μM) for recombinant and native $P2X_7$ (Surprenant *et al*, 1996; Gargett *et al*, 1997). KN-62 at either concentration completely inhibited the ATP-induced ethidium$^+$ uptake into CD1a$^+$ EC isolated from three different subjects (Table I). Similarly, KN-62 at 10 or 1 μM significantly inhibited ATP-induced ethidium$^+$ uptake into CD1a$^-$ EC from these same three subjects by over 98% or 90%, respectively (Table I). Ethidium$^+$ uptake in the absence of ATP was similar in EC incubated with KN-62 or DMSO vehicle (*results not shown*).

Monocyte-derived Langerhans cells (Mo-LC) express $P2X_7$ To further study the expression and function of $P2X_7$ on LC, Mo-LC were generated using a standard protocol (Geissmann *et al*, 2002). The identity of the Mo-LC was confirmed by expression of the LC markers, CD1a, E-cadherin, and CD207 (Langerin), as well as the DC markers, CD11c and HLA-DR, low to medium levels of the co-stimulatory molecules, CD40, CD80, and CD86, and near-to-absent levels of both the DC maturation marker, CD83 and the monocyte/macrophage marker, CD14 (Fig S1; Table S1). Importantly, as observed with epidermal LC, Mo-LC expressed $P2X_7$ (MFI of 10.5 ± 6.6, n = 19), as well as CD39 (MFI of 51.1 ± 24.9, n = 19) (Fig 3).

$P2X_7$ agonists induce ethidium$^+$ uptake into Mo-LC The effect of various extracellular nucleotides on ethidium$^+$ uptake into Mo-LC was then studied. Both 200 μM BzATP and 1 mM ATP induced the uptake of ethidium$^+$ with mean arbitrary units of ethidium$^+$ uptake of 7652 ± 634 and 5630 ± 1223, respectively (n = 3; Fig 4). As for epidermal LC, 1 mM ADP, and 1 mM UTP failed to induce ethidium$^+$ uptake with mean arbitrary units of ethidium$^+$ uptake of 47 ± 43 and 38 ± 52, respectively (n = 3; Fig 4).

$P2X_7$ antagonist impairs ATP-induced ethidium$^+$ uptake into Mo-LC To confirm a role for $P2X_7$ in ATP-induced uptake of ethidium$^+$ into Mo-LC, cells were pre-incubated with KN-62 or an equal volume of DMSO vehicle, followed by incubation with 100 μM ATP. Similar to epidermal LC, KN-62 at a concentration of either 1 or 10 μM significantly inhibited the ATP-induced ethidium$^+$ uptake into Mo-LC by 85.6 ± 6.0% and 98.1 ± 3.3% respectively (n = 3, $p<0.01$; Fig 5). Ethidium$^+$ uptake in the absence of ATP was similar in Mo-LC incubated with KN-62 or DMSO vehicle (*results not shown*).

ATP-induced ethidium$^+$ uptake is impaired in Mo-LC homozygous for the $Glu^{496}Ala$ polymorphism Mo-LC offer the unique advantage in that these cells can be readily generated from subjects with known loss-of-function polymorphisms in $P2X_7$ such as the $Glu^{496}Ala$ polymorphism (Gu *et al*, 2001). Therefore, Mo-LC were generated in paired experiments from subjects who were either wild-type or homozygous for the $Glu^{496}Ala$ polymorphism, and both $P2X_7$ expression and function were examined. Using a small panel of phenotypic markers, there was no difference in the level of expression of either CD1a, CD39, CD83, CD207, or E-cadherin on Mo-LC between genotypes (*results not*

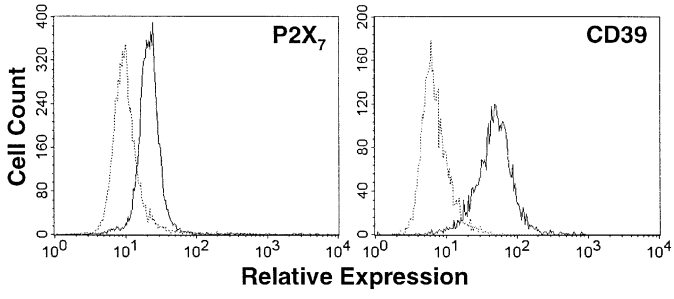

Figure 3
Expression of $P2X_7$ receptors and CD39 on monocyte-derived Langerhans cells (Mo-LC). Mo-LC were labeled with fluorescein isothiocyanate (FITC)-$P2X_7$ (*solid line*) or FITC-isotype control (*dotted line*) monoclonal antibody (mAb), or phycoerythrin (PE)-CD39 (*solid line*) or PE-isotype control (*dotted line*) mAb and analyzed by flow cytometry. Results are representative of 19 experiments.

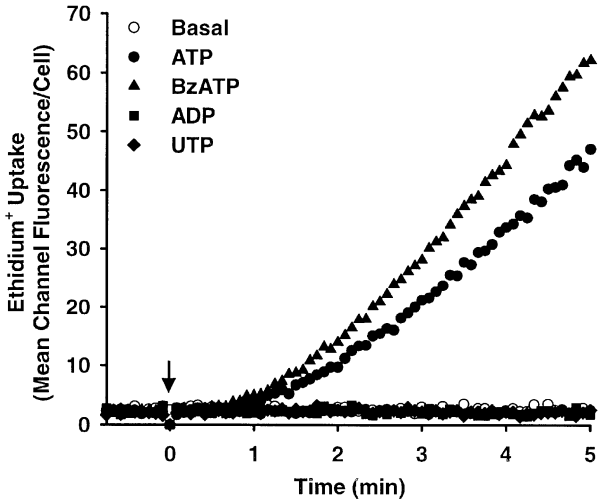

Figure 4
P2X$_7$ agonists induce ethidium$^+$ uptake into monocyte-derived Langerhans cells (Mo-LC). Epidermal cells were labeled with fluorescein isothiocyanate (FITC)-CD1a monoclonal antibody and suspended in KCl medium at 37°C. Ethidium$^+$ (25 μM) was added, followed 40 s later by the addition of nucleotide (*arrow*). The mean channel of cell-associated fluorescence was measured by time-resolved flow cytometry for Mo-LC incubated in the absence (○; basal) or presence of 1 mM ATP (adenosine 5′-triphosphate) (●), 200 μM BzATP (2′- and 3′-0(4-benzoylbenzoyl) ATP) (▲), 1 mM ADP (adenosine 5′-diphosphate) (■), or 1 mM UTP (uridine 5′-triphosphate) (◆). Results are representative of three experiments.

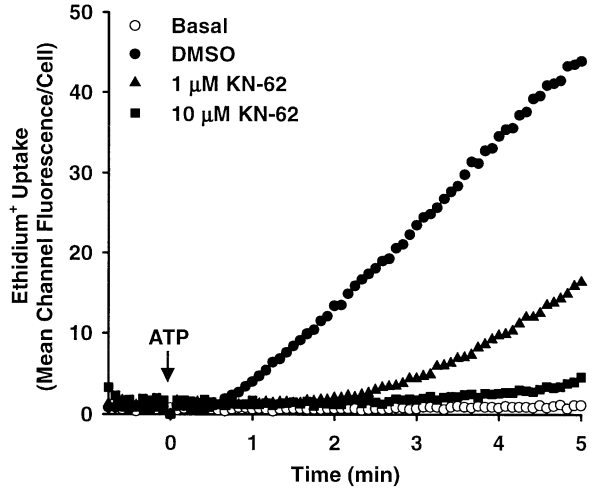

Figure 5
P2X$_7$ antagonist inhibits adenosine 5′-triphosphate (ATP)-induced ethidium$^+$ uptake into monocyte-derived Langerhans cells (Mo-LC). Mo-LC were labeled with fluorescein isothiocyanate (FITC)-CD1a mAb and suspended in KCl medium at 37°C. Mo-LC were pre-incubated with dimethyl sulfoxide vehicle (●), 1 μM KN-62 (1-[*N*,*O*-bis(5-isoquinolinesulphonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine) (▲), or 10 μM KN-62 (■) at 37°C for 15 min; then, 25 μM ethidium$^+$ was added, followed 40 s later by the addition of 100 μM ATP (*arrow*). The mean channel of cell-associated fluorescence was measured by time-resolved flow cytometry for Mo-LC incubated in the absence (○; basal) or presence of ATP (●,▲,■). Results are representative of three experiments.

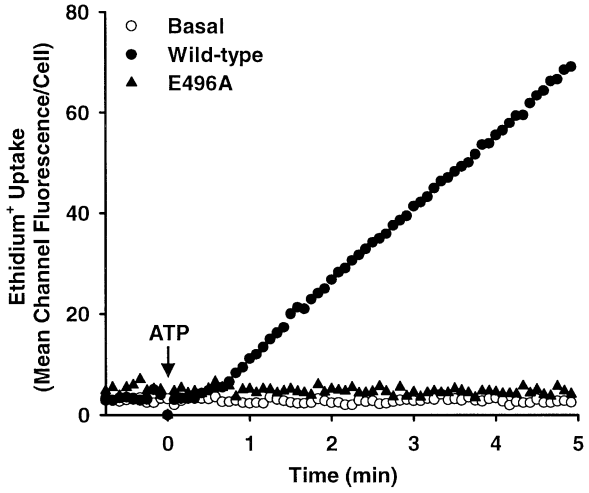

Figure 6
Glu^{496}Ala polymorphism impairs adenosine 5′-triphosphate (ATP)-induced ethidium$^+$ uptake into monocyte-derived Langerhans cells (Mo-LC). Mo-LC from a subjects either wild-type at 496 (○,●) or homozygous for the Glu^{496}Ala (E496A) polymorphism (▲) were labeled with fluorescein isothiocyanate (FITC)-CD1a monoclonial antibody and suspended in KCl medium at 37°C. Ethidium$^+$ (25 μM) was added, followed 40 s later by the addition of 1 mM ATP (*arrow*). The mean channel of cell-associated fluorescence was measured by time-resolved flow cytometry for Mo-LC incubated in the absence (○; basal) or presence (●, ▲) of ATP. Results are representative of three experiments.

shown). In contrast, P2X$_7$ expression was lower on homozygous Mo-LC than on wild-type Mo-LC (MFI of 4.6 ± 8.0 *vs* 15.1 ± 4.0, $n = 3$), although this failed to reach significance ($p = 0.11$). A more striking difference was seen in P2X$_7$ function since ATP-induced ethidium$^+$ uptake was near to absent in homozygous Mo-LC and significantly lower when compared with wild-type Mo-LC (mean arbitrary units of ethidium$^+$ uptake of 34 ± 59 *vs* 13906 ± 5518, respectively, $n = 3$, $p < 0.02$; Fig 6).

P2X$_7$ function in Mo-LC and Mo-DC, and monocytes
P2X$_7$ function increases as monocytes differentiate into macrophages (Hickman *et al*, 1994); however, it has not been reported whether a similar phenomenon occurs as monocytes differentiate into LC or DC. Therefore, to examine this and to compare the relative levels of P2X$_7$ function between these two DC subtypes, Mo-LC and Mo-DC were generated in paired experiments, and the ATP-induced ethidium$^+$ uptake was examined in these cells obtained at day 6, as well as in monocytes present in the peripheral blood mononuclear cell preparations obtained on day 0. ATP induced the uptake of ethidium$^+$ into Mo-DC (mean arbitrary units of uptake of 15148 ± 5149, $n = 3$), which was on average 2-fold higher than that into Mo-LC (mean arbitrary units of uptake of 8316 ± 3764, $n = 3$) (Fig 7). ATP also induced the uptake of ethidium$^+$ into monocytes (mean arbitrary units of uptake of 4759 ± 1543, $n = 3$; Fig 7); however, this was at levels lower than that observed in Mo-LC and Mo-DC, and in the latter case reaching statistical significance ($p < 0.03$).

In these paired experiments, the level of P2X$_7$ and CD39 expression on Mo-LC and Mo-DC was also examined. Mo-LC and Mo-DC expressed similar levels of P2X$_7$ on the cell

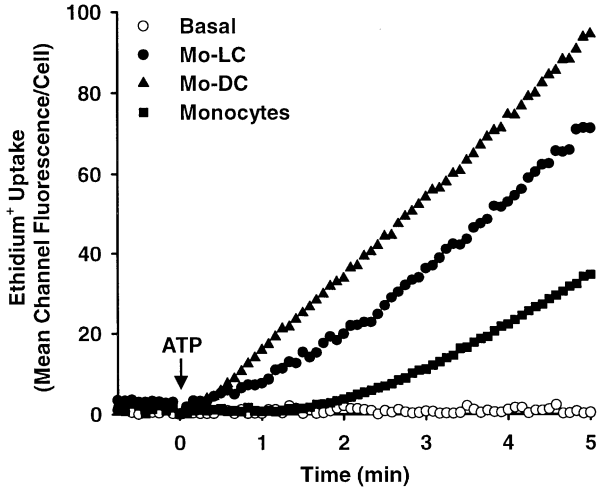

Figure 7
Adenosine 5′-triphosphate (ATP) induced ethidium$^+$ uptake into monocyte-derived Langerhans cells (Mo-LC), monocyte-derived dendritic cells (Mo-DC), and monocytes. Day 6 Mo-LC (○,●) and Mo-DC (▲) were labeled with fluorescein isothiocyanate (FITC)-CD1a monoclonal antibody (mAb), and day 0 monocytes (■) were labeled with FITC-CD14 mAb, and suspended in KCl medium at 37°C. Ethidium$^+$ (25 μM) was added, followed 40 s later by the addition of 1 mM ATP (*arrow*). The mean channel of cell-associated fluorescence was measured by time-resolved flow cytometry for cells incubated in the absence (○; basal) or presence of ATP (●,▲,■). Results are representative of three experiments.

surface (MFI of 12.8 ± 3.5 and 15.2 ± 0.4, respectively, $n = 3$; Fig 8). In contrast, the level of CD39 expressed on Mo-LC was 3-fold greater than that on Mo-DC (MFI of 66.4 ± 23.7 *vs* 24.8 ± 15.0, respectively, $n = 3$; Fig 8), although this failed to reach statistical significance ($p = 0.06$).

ATP induces CD23 shedding from Mo-LC via P2X$_7$ activation Untreated Mo-LC were found to express CD23 (51.1 ± 30.0 MFI, $n = 3$; Fig 9). Therefore, to determine whether activation of P2X$_7$ can also cause downstream signaling events in Mo-LC, the effect of ATP on CD23 shedding was examined as previously described for B lymphocytes (Gu *et al*, 1998) and Mo-DC (Sluyter and Wiley, 2002). CD23 expression on Mo-LC incubated in the absence of nucleotide (Fig 9) was similar to that of untreated cells and was used to determine the percentage loss of CD23 in the presence of nucleotide. Incubation of Mo-LC with either 200 μM BzATP or 1 mM ATP for 5 min induced a near-complete loss of CD23 from these cells (mean loss of CD23 of $87.1 \pm 10.5\%$ and $87.6 \pm 10.3\%$, respectively, $n = 5$; Fig 9). In contrast, 1 mM ADP and 1 mM UTP had a minimal effect (mean loss of CD23 of $10.6 \pm 9.4\%$ and $4.1 \pm 4.1\%$, respectively, $n = 5$; Fig 9).

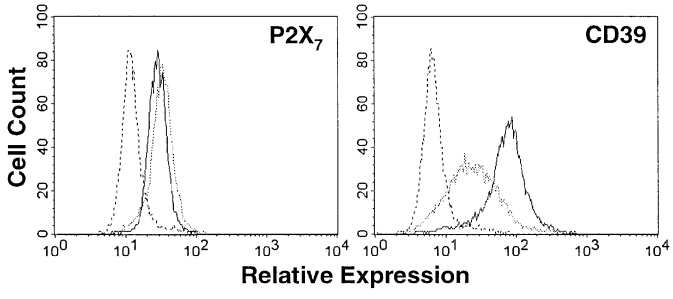

Figure 8
Expression of P2X$_7$ receptors and CD39 on monocyte-derived Langerhans cells (Mo-LC) and monocyte-derived dendritic cells (Mo-DC). Mo-LC (*solid and dotted lines*) and Mo-DC (*shaded line*) were generated in paired experiments and labeled with fluorescein isothiocyanate (FITC)-P2X$_7$ (*solid and shaded lines*) or FITC-isotype control (*dotted line*) monoclonal antibody (mAb), or phycoerythrin (PE)-CD39 (*solid and shaded lines*) or PE-isotype control (*dotted line*) mAb and analyzed by flow cytometry. For clarity, only isotype control mAb labeling on Mo-LC is shown. Results are representative of three experiments.

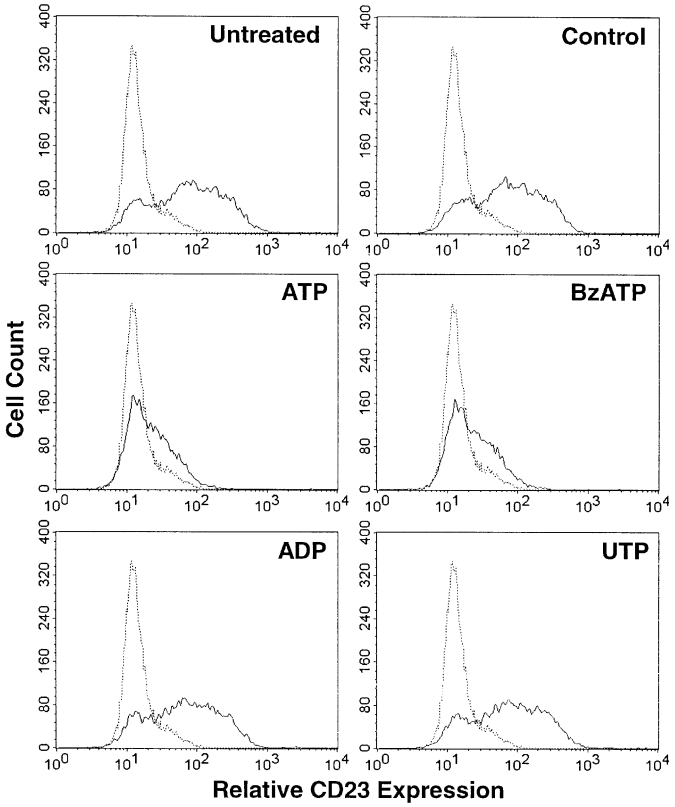

Figure 9
P2X$_7$ agonists induce CD23 shedding from monocyte-derived Langerhans cells (Mo-LC). Mo-LC in KCl medium were incubated in the absence (control) or presence of 1 mM ATP (adenosine 5′-triphosphate), 200 μM BzATP (2′- and 3′-0(4-benzoylbenzoyl) ATP), 1 mM ADP (adenosine 5′-diphosphate), or 1 mM UTP (uridine 5′-triphosphate) at 37°C for 5 min. Incubations were stopped by the addition of $MgCl_2$ medium followed by centrifugation. Untreated or treated Mo-LC were labeled with phycoerythrin (PE)-CD23 (*solid line*) or PE-isotype control (*dotted line*) monoclonal antibody (mAb) and analyzed by flow cytometry. Results are representative of five experiments.

To confirm a role for P2X$_7$ in the ATP-induced shedding of CD23 from wild-type Mo-LC, cells were pre-incubated with KN-62 or an equal volume of DMSO vehicle before incubation with 100 μM ATP. KN-62 at concentrations of either 1 or 10 μM inhibited the ATP-induced shedding of CD23 from Mo-LC by $64.4 \pm 36.4\%$ and $88.0 \pm 20.8\%$, respectively ($n = 3$); however, only the latter reached statistical significance ($p < 0.02$).

The ability of ATP to induce shedding of CD23 from the surface of Mo-LC was also compared using Mo-LC generated from subjects who were wild-type or homozygous for the Glu^{496}Ala polymorphism. Similar to the other phenotypic markers (*above*), the initial level of CD23 on Mo-LC of either

genotype was similar (*results not shown*). In paired experiments, ATP-induced shedding of CD23 was significantly lower from homozygous Mo-LC than from wild-type Mo-LC (mean loss of CD23 of $47.8 \pm 5.0\%$ *vs* $86.1 \pm 6.1\%$, respectively, $n = 3$, $p < 0.01$).

Discussion

The main finding of this study is that human epidermal LC and Mo-LC both express functional P2X$_7$ receptors. Using an anti-P2X$_7$ mAb and flow cytometry, we demonstrated that P2X$_7$ was present on the surface of LC and that ATP induced the uptake of ethidium$^+$ into these cells. Several observations demonstrate that P2X$_7$ is responsible for this ethidium$^+$ uptake. First, the P2X$_7$ agonists BzATP and ATP, but not ADP or UTP (agonists of other P2X and P2Y receptors), induced ethidium$^+$ uptake into LC. Moreover, 200 μM BzATP, a full agonist for the P2X$_7$ receptor (Gargett *et al*, 1997), consistently induced higher levels of ethidium$^+$ uptake than 1 mM ATP, which is a partial P2X$_7$ agonist. Second, the specific P2X$_7$ antagonist, KN-62, inhibited the ATP-induced ethidium$^+$ uptake into LC. Third, ATP-induced ethidium$^+$ uptake was impaired in Mo-LC homozygous for the loss-of-function Glu^{496}Ala polymorphism.

Few studies have determined whether P2X$_7$ is present on tissue-derived DC. Anti-P2X$_7$ mAb labeled DC-like cells in human tonsil (Buell *et al*, 1998) and freshly isolated murine splenic DC express a receptor with functional characteristics of P2X$_7$ (Nihei *et al*, 2000), while ATP can induce uptake of Lucifer Yellow into murine fetal skin-derived DC (Mutini *et al*, 1999) and murine epidermal LC (Mizumoto *et al*, 2002). Moreover, ATP can permeabilize human epidermal LC as assessed by propidium^{2+} uptake via a process characteristic of P2X$_7$ activation (Girolomoni *et al*, 1993). These LC, however, were relatively resistant to the permeabilizing effect of ATP, due to membrane ATPDase activity, with less than 5% of LC positive for propidium^{2+} uptake after 5 min incubation with 10 mM ATP or after a 30-min incubation with 1 mM ATP. In contrast, in our study 1 mM ATP induced the uptake of ethidium$^+$ into $\sim$55% of LC within 5 min. This difference is most likely due to differences in the incubation media and the fluorescent cations used. The previous study incubated LC in serum-free DMEM culture media, whereas our study used a KCl medium that is nominally free of extracellular Na^+, Ca^{2+}, and Mg^{2+}. These cations are all standard constituents of culture media but are known to inhibit P2X$_7$ function by binding to an unidentified inhibitory site on the receptor (Wiley *et al*, 1992; Michel *et al*, 1999) or by reducing the availability of the ATP^{4-} species required for P2X$_7$ activation (North, 2002). In addition, the presence of Ca^{2+} and Mg^{2+} in the culture medium may have enhanced the cation-dependent ATPDase activity of CD39 (Wang and Guidotti, 1996), thus reducing the half-life of extracellular ATP. Differences in permeability have also been reported for ethidium$^+$ and propidium^{2+}. P2X$_7$ pore formation allows uptake of cations up to the size of ethidium$^+$ (314 Da) and YoPRO-1$^+$ (375 Da) but not propidium^{2+} (415 Da) (Wiley *et al*, 1993; Chused *et al*, 1996), whereas ATP-induced uptake of propidium^{2+} and the anion, Lucifer Yellow (457 Da), occurs via non-selective pores (Steinberg *et al*, 1987) and possibly represents an early event of P2X$_7$-mediated apoptosis.

The presence of P2X$_7$ on human Mo-DC has been reported by several groups (Berchtold *et al*, 1999; Coutinho-Silva *et al*, 1999; Ferrari *et al*, 2000; Sluyter and Wiley, 2002). Here, we demonstrate that functional P2X$_7$ receptors are also present on Mo-LC. Mo-LC have been used as a model to study LC by others (Geissmann *et al*, 2002; Guironnet *et al*, 2002; Mizuno *et al*, 2004) and phenotypic analysis of the Mo-LC generated in this study resemble those previously described, expressing the LC markers CD1a$^+$, E-cadherin, and CD207 (Langerin), as well as the DC markers CD11c and HLA-DR. Moreover, our cultured Mo-LC expressed low to moderate levels of the activation markers, CD40, CD80, CD83, and CD86, typical of immature LC within the epidermis. This *in vitro* model allowed the generation of Mo-LC from subjects either wild-type or homozygous for the Glu^{496}Ala polymorphism. ATP has been shown to induce maturation of Mo-DC (Berchtold *et al*, 1999; Schnurr *et al*, 2000), a process thought to be mediated by P2Y$_{11}$ (Wilkin *et al*, 2001). Here, Mo-LC of either genotype had a similar phenotype and thus it is unlikely that P2X$_7$ affects the differentiation or maturation of Mo-LC. The expression of P2X$_7$ on homozygous Mo-LC, however, was lower than that on their wild-type counterparts. Although the Glu^{496}Ala polymorphism does not affect P2X$_7$ expression on lymphocytes (Gu *et al*, 2001), its expression on lipopolysaccharide-primed monocytes and on interferon-γ-activated macrophages generated from homozygous subjects is also reduced (Saunders *et al*, 2003; Sluyter *et al*, 2004a), suggesting that this polymorphism may affect the upregulation or retention of this receptor on the surface of cells of monocyte lineage.

A direct comparison of ATP-induced ethidium$^+$ uptake into epidermal LC and Mo-LC was not possible due to differences in flow cytometer settings; however, the *in vitro* model allowed a comparison of P2X$_7$ function on Mo-LC, Mo-DC, and monocytes. Comparison of ATP-induced ethidium$^+$ uptake demonstrated that P2X$_7$ function was 2- and 3-fold higher, respectively, on Mo-LC and Mo-DC compared with monocytes, which parallels the many-fold upregulation of P2X$_7$ function during monocyte to macrophage differentiation (Hickman *et al*, 1994). Interestingly, P2X$_7$ function was 2-fold higher on Mo-DC than on Mo-LC despite a similar level of P2X$_7$ expression between the two cell types. In contrast, CD39 expression was 3-fold higher on Mo-LC than on Mo-DC, suggesting that P2X$_7$ function on LC may in part be regulated by the ecto-ATPase, CD39.

Extracellular ATP also induced the shedding of the low-affinity receptor for IgE, CD23, from Mo-LC via activation of P2X$_7$. BzATP and ATP, but neither ADP nor UTP, induced CD23 shedding from Mo-LC, a process inhibited by KN-62 and impaired in Mo-LC homozygous for the loss-of-function Glu^{496}Ala polymorphism. An additional role for other P2X receptors in the ATP-induced shedding of CD23 from Mo-LC, however, cannot be excluded as this ATP-induced shedding was not completely inhibited by KN-62 or in Mo-LC homozygous for the Glu^{496}Ala polymorphism, and that mRNA for P2X$_{1,4,5}$ is expressed in monocytes and Mo-DC (Berchtold *et al*, 1999; Ferrari *et al*, 2000; Wang *et al*, 2004). Nevertheless, given the potential role of CD23 in allergy and

inflammation (Kijimoto-Ochiai, 2002), ATP-induced shedding of CD23 from LC in the skin may have a role in health and disease.

The ecto-ATPDase, CD39, was also found on epidermal LC and Mo-LC but not on keratinocytes. The presence of ATPDase activity on epidermal LC has been known for close to 40 y (Wolf and Winkelmann, 1967) and has been used as a phenotypic marker for these cells (Romani *et al*, 2003). But not until recent studies using CD39 knockout mice was this ATPDase activity identified as CD39 (Mizumoto *et al*, 2002). In contrast, expression of CD39 on human LC is essentially unknown. To the best of our knowledge, only one other study has reported the expression of CD39 on $CD1a^+$ LC in the human epidermis (Davis *et al*, 1988), whereas CD39 mRNA has been identified in Mo-DC (Berchtold *et al*, 1999). Although our study did not examine the activity of CD39 on LC, the presence of CD39 on both epidermal and Mo-LC supports the hypothesis that CD39 is the predominant ATPDase on human LC.

Functional $P2X_7$ was also present on keratinocytes. As for epidermal LC, anti-$P2X_7$ mAb labeled these keratinocytes, and BzATP and ATP, but neither ADP nor UTP, induced $ethidium^+$ uptake into these cells, a process that could be inhibited by KN-62. Immunohistochemistry and functional studies have demonstrated the presence of $P2X_7$ on rodent and human keratinocytes (Groschel-Stewart *et al*, 1999; Greig *et al*, 2003; Inoue *et al,* 2005), where it may play a role in terminal differentiation and/or apoptosis (Greig *et al*, 2003; Inoue *et al,* 2005). Moreover, ATP can permeabilize human keratinocytes as assessed by $propidium^{2+}$ uptake via a molecule characteristic of $P2X_7$ and to a level greater than that observed for epidermal LC (Girolomoni *et al*, 1993). Consistent with this observation, in our study, the BzATP- and ATP-induced $ethidium^+$ uptake into keratinocytes was also significantly greater than into epidermal LC.

The functional significance of $P2X_7$ on LC is unknown; however, the studies with CD39 knockout mice support multiple roles for extracellular ATP in the skin immune system. Contact hypersensitivity (allergic contact dermatitis) is attenuated in CD39 knockout mice, whereas chemically induced cutaneous inflammation (irritant contact dermatitis) is exacerbated in these mice possibly due to desensitization of P2Y and activation of P2X receptors, respectively, as a result of excessive amounts of extracellular ATP (Mizumoto *et al*, 2002). Since ATP via $P2X_7$ activation is known to induce the release of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-18, and tumor necrosis factor from monocytes and Mo-DC (Ferrari *et al*, 2000; Sluyter *et al*, 2004a, c), $P2X_7$ on LC may play a pro-inflammatory role in response to irritant chemicals known to cause ATP release from keratinocytes (Mizumoto *et al*, 2002, 2003). It is interesting to speculate on how the pro-inflammatory role for $P2X_7$ in the skin may be modulated. Water from the Dead Sea, which is particularly rich in Mg^{2+} ions, has inhibitory functions on epidermal LC (Schempp *et al*, 2000) and since Mg^{2+} can reduce the availability of the ATP^{4-} species necessary to activate $P2X_7$ (North, 2002), some of the therapeutic properties of treatments using salts from the Dead Sea may involve inhibition of $P2X_7$. Nevertheless, further studies to determine the function of $P2X_7$ on epidermal LC and the role of extracellular ATP in the skin immune system are warranted.

Materials and Methods

Reagents Nucleotides, RPMI-1640 medium, gentamycin, bovine serum albumin, DMSO, 7-aminoactinomycin D (7AAD), and ethidium bromide were from Sigma Chemica (St Louis, Missouri). KN-62 was from BIOMOL (Plymouth Meeting, Pennsylvania). Fetal calf serum, GlutaMAX-1, non-essential amino acids, 2-mercaptoethanol, and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were from Invitrogen (Auckland, New Zealand), and polymerase chain reaction reagents were from Invitrogen (Carlsbad, California). Ficoll–Paque PLUS was from Amersham Biosciences (Uppsala, Sweden). Dispase II, trypsin, and DNase I were from Roche Diagnostics (Mannheim, Germany). Granulocyte macrophage colony-stimulating factor (GM-CSF) was a generous gift from Schering-Plough (Baulkham Hills, Australia). IL-4 and transforming growth factor-β1 (TGFβ1) were from R&D Systems (Minneapolis, Minnesota). Fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-anti-CD1a monoclonal antibodies (mAb), FITC-conjugated anti-CD80 and anti-CD86 mAb, PE-conjugated anti-CD11c, anti-CD23, anti-CD39, anti-CD40 and anti-CD83 mAb, and PerCP-conjugated anti-HLA-DR mAb were from BD Biosciences (San Diego, California). PE-conjugated anti-CD207 (anti-Langerin) mAb was from Beckman-Coulter (Fullerton, California). Anti-E-cadherin mAb was from Zymed Laboratories (South San Francisco, California). FITC- and PE-conjugated anti-CD14 mAb were from Dako (Carpinteria, California). FITC-conjugated anti-$P2X_7$ receptor was prepared as described (Buell *et al*, 1998). Sheep PE-conjugated anti-mouse Ig F(ab′)2 fragment was from Chemicon (Melbourne, Australia).

Samples Studies were performed according to the Declaration of Helsinki Principles, and were approved by the Wentworth Area Health Service Human Ethics Committee (Penrith, Australia). Samples were collected with informed written consent. Skin (up to 0.5×3 cm) was excised from the abdomen of six patients undergoing routine surgery for various gastrointestinal problems at Nepean Hospital (Penrith, Australia). Peripheral blood (50 mL) was collected in heparin-containing vacutainer tubes from 16 healthy subjects of unknown or known *P2X7* genotype (Gu *et al*, 2001, 2004; Wiley *et al*, 2003; Skarratt *et al,* 2005).

DNA isolation and *P2X7* genotyping Genomic DNA was isolated from whole blood of subjects with unknown *P2X7* genotype using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, Wisconsin) according to the manufacturer's instructions. Exons 1 (including the 5′ boundary of intron 1), 9, and 13 of the *P2X7* gene were amplified from genomic DNA by the polymerase chain reaction and the products were sequenced as described (Gu *et al*, 2004).

Isolation of EC EC were isolated as described (Lynch *et al*, 2003). Briefly, skin pieces were incubated in RPMI-1640 medium containing 0.5% dispase II and 10 mM HEPES for 18 h at 4°C. The resulting epidermal sheets were incubated in phosphate-buffered saline (PBS) containing 0.3% trypsin for 20 min at 37°C, before adding an equal volume of RPMI-1640 medium containing 10% heat-inactivated fetal calf serum, 0.03% DNase I and 10 mM HEPES and mechanically agitating for 5 min. The EC suspension was then filtered through a 70 μm cell strainer, centrifuged, and the EC were resuspended in NaCl medium (145 mM NaCl, 5 mM KCl, 5 mM D-glucose, 0.1% bovine serum albumin, 10 mM HEPES, pH 7.5).

Mo-LC and Mo-DC Mo-LC and Mo-DC were generated as previously described (Geissmann *et al*, 2002; Sluyter and Wiley, 2002). Peripheral blood mononuclear cells were separated by gradient centrifugation using Ficoll–Paque PLUS, and cultured for 2 h at 37°C and 95% air/5% CO_2 in RPMI-1640 containing 10% heat-

inactivated fetal calf serum, 2 mM GlutaMAX-1, 0.1 mM non-essential amino acids, 55 μM 2-mercaptoethanol, 5 μg per mL gentamycin, and 10 mM HEPES. The plastic-adherent monocytes were then cultured in complete media (containing 800 U per mL GM-CSF and 500 U per mL IL-4) in the presence or absence of 10 ng per mL TGFβ1 to generate Mo-LC and Mo-DC, respectively. On day 3, 50% of the medium was replaced with an equal volume of complete media containing GM-CSF and either TGFβ1 for Mo-LC or IL-4 for Mo-DC. On day 6, the non-adherent cells were collected and resuspended in NaCl medium. Flow cytometric analysis of various molecules on the cell surface of Mo-LC indicated that 72%–99% expressed CD1a, 1%–60% expressed CD207 (Langerin), and 37%–92% expressed E-cadherin. A similar analysis of the Mo-DC indicated that 65%–99% expressed CD1a, 0%–9% expressed CD207, and 0%–30% expressed E-cadherin. Additional details of the Mo-LC and Mo-DC phenotypes can be obtained from the online supplementary materials (Fig S1, Table S1).

Immunolabeling and flow cytometry Cells were labeled with fluorophore-conjugated mAb in NaCl medium containing 10% human AB serum and 0.01% NaN_3, and then washed once with PBS. For E-cadherin staining, cells were labeled with unconjugated E-cadherin or isotype control mAb in NaCl medium containing 10% human AB serum, 1 mM $CaCl_2$ and 0.01% NaN_3, washed twice with NaCl medium, incubated with sheep PE-conjugated anti-mouse Ig $F(ab')_2$ fragment in NaCl medium, and then washed once with PBS. Cells were examined using a Becton Dickinson (San Jose, California) FACSCalibur flow cytometer collecting a total of 150–200 $\times$ 10^3 events for EC, 5–10 $\times$ 10^3 events for Mo-LC and Mo-DC, and 40 $\times$ 10^3 events for peripheral blood mononuclear cells. Cells were analyzed in the log mean channel of fluorescence intensity for each gated population using CellQuest Software (Becton Dickinson). The level of cell surface expression of each molecule was determined as the MFI of specific mAb binding minus the MFI of the appropriate isotype control mAb binding.

Measurement of ethidium$^+$ uptake into epidermal cells EC in 1 mL KCl medium (150 mM KCl, 5 mM D-glucose, 0.1% bovine serum albumin, 10 mM HEPES, pH 7.5) containing 25 μM ethidium$^+$ were incubated for 5 min at 37°C in the presence or absence of nucleotide as indicated. In some experiments, cells were pre-incubated for 15 min at 37°C in the presence of the P2X$_7$ antagonist, KN-62 (Gargett and Wiley, 1997), or an equal volume of DMSO vehicle. Incubations with nucleotide were terminated by adding 1 mL of ice-cold 20 mM $MgCl_2$ medium (20 mM $MgCl_2$, 145 mM NaCl, 5 mM KCl and 10 mM HEPES, pH 7.5) and centrifugation. The cells were then washed with PBS, labeled with FITC-conjugated anti-CD1a mAb, and the level of ethidium$^+$ uptake was determined by flow cytometry. Cells were analyzed in the log mean channel of fluorescence intensity for each gated population using CellQuest Software. The level of nucleotide-induced ethidium$^+$ uptake was determined as the difference in MFI of cell-associated ethidium$^+$ of cells incubated in the presence and absence of nucleotide.

Measurement of ethidium$^+$ uptake by time-resolved flow cytometry Nucleotide-induced ethidium$^+$ influx into Mo-LC, Mo-DC, and peripheral blood mononuclear cells was measured by time-resolved flow cytometry as previously described (Sluyter and Wiley, 2002). Briefly, cells were pre-labeled with FITC-conjugated anti-CD1a or anti-CD14 mAb and resuspended in 1 mL KCl medium at 37°C. At 0 s, 25 μM ethidium$^+$ was added, followed 40 s later by the addition of nucleotide as indicated. In some experiments, cells were pre-incubated for 15 min at 37°C in the presence of KN-62 or an equal volume of DMSO vehicle. Data were collected by flow cytometry over 6 min at 37°C with constant stirring using a Cytek (Fremont, California) Time Zero Module. The linear mean channel of ethidium$^+$ fluorescence intensity for each gated population over successive 5 s intervals was analyzed by WinMDI 2.8 Software developed by Joseph Trotter (http://www.scripps.edu) and plotted against time. Nucleotide-induced ethidium$^+$ uptake was quantitated as the difference in arbitrary units of area under the uptake curves in the presence and absence of nucleotide in the first 5 min of incubation.

Measurement of CD23 shedding Mo-LC in 1 mL KCl medium were incubated for 5 min at 37°C in the presence or absence of extracellular nucleotide as indicated. In some experiments, cells were pre-incubated for 15 min at 37°C in the presence of KN-62 or an equal volume of DMSO vehicle. Incubations with nucleotide were terminated by adding 1 mL of ice-cold 20 mM $MgCl_2$ medium and centrifugation. The cells were then washed with PBS, and the cell surface expression of CD23 was determined using immunolabeling and flow cytometry. Results are presented as the percentage loss of CD23 expression (MFI) in the presence of nucleotide compared with CD23 expression (MFI) in the absence of nucleotide.

Presentation of data and statistics Data are presented as mean ± SD. Comparisons between groups were performed using the two-tailed Student's unpaired *t* test and was considered significant when $p < 0.05$.

This study was supported by the National Health & Medical Research Council of Australia and the Leukemia Foundation of New South Wales. We gratefully acknowledge Ms. Mary Tiller and Ms. Pam Craig for assistance in obtaining skin samples, Schering-Plough for generously providing GM-CSF, Dr. Ben Gu for preparing the anti-P2X$_7$ mAb, and Ms. Lan-Phuong Dao-Ung for helpful advice regarding flow cytometry.

Supplementary Material

The following supplementary material is available for this article online.
Figure S1 Expression of phenotypic molecules on Mo-LC and Mo-DC.
Table S1 Phonotype of Mo-LC and Mo-Dc.

DOI: 10.1111/j.0022-202X.2005.23835.x

Manuscript received February 1, 2005; revised March 21, 2005; accepted for publication April 14, 2005

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References

Berchtold S, Ogilvie ALJ, Bogdan C, Muhl-Zurbes P, Ogilvie A, Schuler G, Steinkasserer A: Human monocyte derived dendritic cells express functional P2X and P2Y receptors as well as ecto-nucleotidases. FEBS Lett 458:424–428, 1999

Buell G, Chessell IP, Michel AD, *et al*: Blockade of human P2X$_7$ receptor function with a monoclonal antibody. Blood 92:3521–3528, 1998

Cavanagh LL, Von Andrian UH: Travellers in many guises: The origins and destinations of dendritic cells. Immunol Cell Biol 80:448–462, 2002

Chused TM, Apasov S, Sitkovsky M: Murine T lymphocytes modulate activity of an ATP-activated P2Z-type purinoceptor during differentiation. J Immunol 157:1371–1380, 1996

Coutinho-Silva R, Persechini PM, Bisaggio RDC, *et al*: P$_{2Z}$/P2X$_7$ receptor-dependent apoptosis of dendritic cells. Am J Physiol Cell Physiol 276: C1139–C1147, 1999

Davis AL, McKenzie JL, Hart DNJ: HLA-DR-positive leucocyte subpopulations in human skin include dendritic cells, macrophages, and CD7-negative T cells. Immunol 65:573–581, 1988

Ferrari D, La Sala A, Chiozzi P, *et al*: The P2 purinergic receptors of human dendritic cells: Identification and coupling to cytokine release. FASEB J 14:2466–2476, 2000

Gargett CE, Cornish JE, Wiley JS: ATP, a partial agonist for the P2Z receptor of human lymphocytes. Br J Pharmacol 122:911–917, 1997

Gargett CE, Wiley JS: The isoquinoline derivative KN-62 a potent antagonist of the P2Z-receptor of human lymphocytes. Br J Pharmacol 120:1483–1490, 1997

Geissmann F, Dieu-Nosjean MC, Dezutter C, *et al*: Accumulation of immature Langerhans cells in human lymph nodes in draining chronically inflamed skin. J Exp Med 196:417–430, 2002

Girolomoni G, Santantonio ML, Pastore S, Bergstresser PR, Giannetti A, Cruz PD: Epidermal Langerhans cells are resistant to the permeabilizing effects of extracellular ATP: *In vitro* evidence supporting a protective role of membrane ATPase. J Invest Dermatol 100:282–287, 1993

Granstein RD: The skinny on CD39 in immunity and inflammation. Nature Med 8:336–338, 2002

Greig AVH, Linge C, Terenghi G, McGrouther DA, Burnstock G: Purinergic receptors are part of a functional signaling system for proliferation and differentiation of human epidermal keratinocytes. J Invest Dermatol 120:1007–1015, 2003

Groschel-Stewart U, Bardini M, Robson T, Burnstock G: Localisation of $P2X_5$ and $P2X_7$ receptors by immunohistochemistry in rat stratified squamous epithelia. Cell Tissue Res 296:599–605, 1999

Gu B, Bendall LJ, Wiley JS: Adenosine triphosphate-induced shedding of CD23 and L-selectin (CD62L) from lymphocytes is mediated by the same receptor but different metalloproteases. Blood 92:946–951, 1998

Gu BJ, Zhang WY, Bendall LJ, Chessell IP, Buell GN, Wiley JS: Expression of $P2X_7$ purinoceptors on human lymphocytes and monocytes: Evidence for nonfunctional P2X7 receptors. Am J Physiol Cell Physiol 279: C1189–C1197, 2000

Gu BJ, Zhang WY, Worthington RA, *et al*: A Glu-496 to Ala polymorphism leads to loss of function of the human $P2X_7$ receptor. J Biol Chem 276: 11135–11142, 2001

Gu BJ, Sluyter R, Skarratt KK, *et al*: An Arg^{307} to Gln polymorphism within the ATP binding site causes loss-of-function of the human $P2X_7$ receptor. J Biol Chem 279:31287–31295, 2004

Guironnet G, Dezutter-Dambuyant C, Vincent C, Bechetoille N, Schmitt D, Peguet-Navarro J: Antagonistic effects of IL-4 and TGF-β1 on Langerhans cell-related antigen expression by human monocytes. J Leuk Biol 71: 845–853, 2002

Hickman SE, El Khoury J, Greenberg S, Schieren I, Silverstein SC: P2Z adenosine triphosphate receptor activity in cultured human monocyte-derived macrophages. Blood 84:2452–2456, 1994

Holzer AM, Granstein RD: Role of extracellular adenosine triphosphate in human skin. J Cut Med Surg 8:90–96, 2004

Inoue K, Denda M, Tozaki H, Fujishita K, Koizumi S, Inoue K: Characterization of multiple P2X receptors in cultured normal human epidermal keratinocytes. J Invest Dermatol 124:756–763, 2005

Kijimoto-Ochiai S: CD23 (the low-affinity IgE receptor) as a C-type lectin: A multidomain and multifunctional molecule. Cell Mol Life Sci 59:648–664, 2002

la Sala A, Ferrari D, Di Virgilio F, Idzko M, Norgauer J, Girolomoni G: Alerting and tuning the immune response by extracellular nucleotides. J Leuk Biol 73:339–343, 2003

Lammas DA, Stober C, Harvey CJ, Kendrick N, Panchalingam S, Kumararatne DS: ATP-induced killing of Mycobacteria by human macrophages is mediated by purinergic P2Z(P2X7) receptors. Immunity 7:433–444, 1997

Lynch GW, Slaytor EK, Elliot FD, *et al*: CD4 is expressed by epidermal Langerhans' cells predominantly as covalent dimers. Exp Dermatol 12: 700–711, 2003

Michel AD, Chessell IP, Humphrey PPA: Ionic effects on human recombinant $P2X_7$ receptor function. Naunyn-Schmiedeberg's Arch Pharmacol 359:102–109, 1999

Mizumoto N, Kumamoto T, Robson SC, Sevigny J, Matsue H, Enjyoji K, Takashima A: CD39 is the dominant Langerhans cell associated ecto-NTPDase: Modulatory roles in inflammation and immune responsiveness. Nat Med 8:358–365, 2002

Mizumoto N, Mummert ME, Shalhevet D, Takashima A: Keratinocyte ATP release assay for testing skin-irritating potentials of structurally diverse chemicals. J Invest Dermatol 121:1066–1072, 2003

Mizuno K, Okamoto H, Horio T: Ultraviolet B radiation suppresses endocytosis, subsequent maturation, and migration activity of Langerhans cell-like dendritic cells. J Invest Dermatol 76:300–306, 2004

Mutini C, Falzoni S, Ferrari D, *et al*: Mouse dendritic cells express the $P2X_7$ purinergic receptor: Characterization and possible participation in antigen presentation. J Immunol 163:1958–1965, 1999

Nihei OK, de Carvalho ACC, Savino W, Alves LA: Pharmacologic properties of P2Z/P2X7 receptor characterized in murine dendritic cells: Role on the induction of apoptosis. Blood 96:996–1005, 2000

North RA: Molecular physiology of P2X receptors. Physiol Rev 82:1013–1067, 2002

Ralevic V, Burnstock G: Receptors for purines and pyrimidines. Pharmacol Rev 50:413–492, 1998

Romani N, Holzmann S, Tripp CH, Koch F, Stoitzner P: Langerhans cells—dendritic cells of the epidermis. APMIS 111:725–740, 2003

Saunders BM, Fernando SL, Sluyter R, Britton WJ, Wiley JS: A loss-of-function polymorphism in the human $P2X_7$ receptor abolishes ATP mediated killing of mycobacteria. J Immunol 171:5442–5446, 2003

Schempp CM, Dittmar HC, Hummler D, Simon-Haarhaus B, Schulte-Monting J, Schopf E, Simon JC: Magnesium ions inhibit the antigen-presenting function of human epidermal Langerhans cells *in vivo* and *in vitro*, involvement of ATPase, HLA-DR, B7 molecules, and cytokines. J Invest Dermatol 115:680–686, 2000

Schnurr M, Then F, Galambos P, Scholz C, Siegmund B, Endres S, Eigler A: Extracellular ATP and TNF-a synergize in the activation and maturation of human dendritic cells. J Immunol 165:4704–4709, 2000

Skarratt KK, Fuller SJ, Sluyter R, Dao-Ung LP, Gu BJ, Wiley JS: A 5′ intronic splice site polymorphism leads to a null allele of the $P2X_7$ gene in 1% to 2% of the Caucasian population. FEBS Lett 579:2675–2678, 2005

Sluyter R, Dalitz JG, Wiley JS: $P2X_7$ receptor polymorphism impairs extracellular adenosine 5′-triphosphate-induced interleukin-18 release from human monocytes. Genes Immunity 5:588–591, 2004a

Sluyter R, Shemon AN, Barden JA, Wiley JS: Extracellular ATP increases cation fluxes in human erythrocytes by activation of the $P2X_7$ receptor. J Biol Chem 279:44749–44755, 2004b

Sluyter R, Shemon AN, Wiley JS: Glu^{496} to Ala polymorphism in the $P2X_7$ receptor impairs ATP-induced IL-1β release from human monocytes. J Immunol 172:3399–3405, 2004c

Sluyter R, Wiley JS: Extracellular adenosine 5′-triphosphate induces a loss of CD23 from human dendritic cells via activation of $P2X_7$ receptors. Int Immunol 14:1415–1421, 2002

Steinberg TH, Newman AS, Swanson JA, Silverstein SC: ATP^{4-} permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes. J Biol Chem 262:8884–8888, 1987

Surprenant A, Rassendren F, Kawashima E, North RA, Buell G: The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). Science 272:735–738, 1996

Wang L, Jacobsen SEW, Bengtsson A, Erlinge D: P2 receptor mRNA expression profiles in human lymphocytes, monocytes and CD34+ stem and progenitor cells. BMC Immunol 5:16, 2004

Wang T-F, Guidotti G: CD39 is an ecto-(Ca^{2+},Mg^{2+})-apyrase. J Biol Chem 271:9898–9901, 1996

Wiley JS, Chen R, Jamieson GP: The ATP^4-receptor-operated channel (P2Z class) of human lymphocytes allows Ba^{2+} and ethidium$^+$ uptake: Inhibition of fluxes by suramin. Arch Biochem Biophys 305:54–60, 1993

Wiley JS, Chen R, Wiley MJ, Jamieson GP: The ATP^{4-} receptor-operated ion channel of human lymphocytes: Inhibition of ion fluxes by amiloride analogs and by extracellular sodium ions. Arch Biochem Biophys 292: 411–418, 1992

Wiley JS, Dao-Ung LP, Li C, *et al*: An Ile-568 to Asn polymorphism prevents normal trafficking and function of the human $P2X_7$ receptor. J Biol Chem 278:17108–17113, 2003

Wilkin F, Duhant X, Bruyns C, Suarez-Huerta N, Boeynaems JM, Robaye B: The $P2Y_{11}$ receptor mediates the ATP-induced maturation of human monocyte-derived dendritic cells. J Immunol 166:7172–7177, 2001

Wolf K, Winkelmann RK: Ultrastructural localization of nucleoside triphosphatase in Langerhans cells. J Invest Dermatol 18:496–500, 1967